

Daily QC – to be performed by the first user of the day

Please check in the PPMS calendar whether you are the first user of the day.

If so, you **have to** run daily QC using SpectroFlo QC beads prior to acquiring samples to ensure that the cytometer is performing optimally. During QC, laser delays and area scaling factors are optimized and gain settings are adjusted automatically to account for day-to-day instrument variability. Note that failure to follow the best practices for QC will negatively impact the quality/comparability of your data.

1. Allow **60 minutes warmup time** after turning on the system to ensure the optics components are warmed up. During this time, run one *Clean Flow Cell* routine, and then dH₂O as a sample at high speed for at least 10 min (open a default experiment from **Acquisition** menu).
2. Diluted QC beads are **stable for 1 day** in the fridge. Discard any older diluted beads.
3. Vortex the SpectroFlo QC beads vial and invert it several times before use. Prepare 1 drop of beads (no more!!!) in 0.3 mL in a 5mL FACS tube (dH₂O is used as sheath solution as well). Label this tube with the **date and “QC”**. Store them in the fridge after QC.
4. Select **QC & Setup** from the Get started menu.
5. Verify/select the current **bead lot number** from the Bead Lot menu.
6. Load a tube of the QC beads onto the SIP. Select **Start** to begin acquisition.
7. The procedure takes app. 3 to 5 minutes to complete. Once complete, remove the tube. Two SIT Flushes are automatically performed to clear the beads from the sample line.
8. A message is displayed when Daily QC is finished. To view the QC report, click View Report; otherwise close the message window and proceed with your experiment.
9. If QC **fails**, follow the instructions in the daily QC Failed dialog that appears, and check the **Troubleshooting guidelines**, and/or contact the Core Facility staff. If not possible at that time, please enter an ‘Incident’ in the PPMS system. Please also let us know in case you need to repeat QC several times before it passes.



Troubleshooting guidelines*:

| Observation | Possible Causes | Recommended Solutions |
|----------------------------|--|--|
| Daily QC does not complete | Wrong QC bead sample | Ensure you are running SpectroFlo QC beads. |
| | Bead sample not properly mixed | Mix the bead sample. |
| | Bead sample too dilute | Concentrate the bead sample or prepare a fresh bead sample. |
| | Air bubble is sample line | Run a SIT Flush. |
| Daily QC failed | Air bubble in fluidics | Run a Purge Filter. |
| | Dirty flow cell | Run a Clean Flow Cell. If the problem persists, run a Clean Flow Cell using 25%-50% Contrad 70, followed by DI water. |
| | Questionable sample prep | Verify the sample prep technique. |
| | Air in sheath filter | Run a Purge Filter. |
| | Sample not diluted in same fluid as sheath | Dilute the sample in the same fluid as the sheath solution. |



*screenshoted from Cytek Aurora Manual 1